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Seroreactivity to specific antigens of *Helicobacter pylori* infection is associated with an increased risk of the dyspeptic gastrointestinal diseases

Chariya Chomvarin [a,*], Orawan Ottiwet [a], Chariya Hahnvajanawong [a], Pewpan M. Intapan [b], Suwin Wongwajana [a]

[a] *Department of Microbiology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*
[b] *Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand*

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Summary
Objectives: The correlation between seroreactivity to *Helicobacter pylori*-specific antigens and clinical outcomes in gastrointestinal disease remains unresolved. We investigated the anti-*H. pylori* antibody profile in northeast Thai dyspeptic patients with gastrointestinal disease in order to identify any *H. pylori* antigens that may be associated with an increased risk of gastrointestinal disease.
Patients and methods: Eighty-nine *H. pylori*-infected dyspeptic patients (44 non-ulcer, 23 peptic ulcer, 22 gastric cancer) were included in the study. Patients were considered to have *H. pylori* infection when at least one invasive method (i.e., culture, rapid urease test, and histology on biopsy specimens) and serological tests including a commercial ELISA (Pyloriset EIA-GIII) and a commercial immunoblot (Helicoblot 2.1; Genelabs Diagnostics), were positive. In addition, the sera of 20 *H. pylori*-infected blood donors and 10 *H. pylori*-non-infected blood donors were also randomly collected and analyzed for *H. pylori* infection by ELISA and Helicoblot 2.1.
Results: Immunoreactive protein bands at 116-kDa, 89-kDa, 37-kDa, 35-kDa, 30-kDa, 19.5-kDa, and the current infection marker for *H. pylori*-infected patients had average frequencies of 97.8%, 77.5%, 36.0%, 25.8%, 79.8%, 58.4%, and 69.7%, respectively. The immunoreactive patterns obtained from the *H. pylori*-infected patients and *H. pylori*-infected blood donors were similar. The antibodies to VacA and CagA antigens were not significantly different among the *H. pylori*-infected gastroduodenal patient groups. The simultaneous presence of antibody to 19.5-kDa antigen and absence of antibody to 35-kDa antigen was associated with an increased risk of gastric cancer ($p < 0.05$). The immunoreactive band to 35-kDa antigen was found at significantly higher levels in peptic ulcer patients, and the 37-kDa antigen was found at significantly higher levels in

* Corresponding author. Tel.: +66 43 363808; fax: +66 43 348385.
E-mail address: chariya@kku.ac.th (C. Chomvarin).

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non-ulcer patients (both $p < 0.05$). Significantly low levels of antibodies to 23-kDa and 85-kDa antigens were found associated with peptic ulcer ($p < 0.05$).
Conclusions: We confirm that the universal presence of CagA and VacA in *H. pylori*-infected patients in Thailand is independent of the gastroduodenal disease. The presence or absence of antibodies to *H. pylori*-specific antigens may be useful as indirect markers in the screening of *H. pylori*-infected patients, and may have specific protection roles in *H. pylori*-related gastroduodenal diseases.

Introduction

Helicobacter pylori is accepted as an important pathogenic factor in human non-ulcer dyspepsia, peptic ulcer dyspepsia, and gastric carcinoma.[1,2] The clinical outcome of *H. pylori* infection shows marked variation, due mainly to diversity in the strain, environmental factors, and host response.[3,4] Since clinical outcomes depend on both the characteristics of the strain and the host response, the antibody response to *H. pylori* could provide clues for predicting the severity of *H. pylori*-associated diseases.[5]

The humoral immune response against *H. pylori* has, however, been reported to show a high polymorphism, which may reflect either an evolution of the immune response or an antigenic shift of the infecting strain.[6] This antibody polymorphism could be related to pathological status and thus may serve as a biological predictor of the gastroduodenal disease in the *H. pylori*-infected.

Many major surface-exposed or surface-secreted antigens, such as the cytotoxin-associated protein (CagA), the vacuolating cytotoxin protein (VacA), urease subunits (UreA and UreB), heat-shock protein (HspA and HspB), flagellin subunits, catalase, lipopolysaccharide, and several undefined antigens, have been studied.[7]

The important putative virulence factors of *H. pylori*, including CagA (a product of the *cagA* gene) and VacA (a product of the *vacA* gene), have been considered to contribute to the risk of developing different *H. pylori*-related outcomes in Western countries.[8,9] However, several studies in Asia, including Thailand, have shown that the presence of the *cagA* gene and the different types of *vacA* are not related to clinical outcomes, as almost all *H. pylori* strains in Thailand are *cagA*-positive and of *vacA*s1 genotype.[10] Thus, the prevalence of these individual antigens (VacA and CagA) has little or no value in predicting the clinical outcome in many countries in Asia.[11–15]

It has been suggested that the low molecular weight (19.5–35-kDa) proteins play an important role in the pathogenesis of *H. pylori*-related disease.[5,15,16] Furthermore, it has been reported that the combination of the presence of seroreactivity to 19.5-kDa antigen and the absence of seroreactivity to 35-kDa antigen is associated with gastric cancer in Singapore.[17] The simultaneous presence of CagA and 26.5-kDa antigen related to peptic ulcer has been reported in Taiwan,[11] and Shiesh et al. found a significant association between the serological response to 19.5-kDa and 26.5-kDa antigens and gastric cancer in Taiwan.[14]

The inconsistency of previous reports makes it necessary to determine whether the association between the serological response to *H. pylori* proteins and specific gastroduodenal diseases depends on geographic distribution, and whether the antigens are related to the type of gastroduodenal disease. These may be useful as markers in screening gastroduodenal diseases and in vaccine development.

The aim of this study was to investigate the anti-*H. pylori* antibody profile by immunoblot assay (Helicoblot 2.1; HB 2.1) in order to correlate the *H. pylori* antigens to the clinical outcomes of gastroduodenal diseases in a Thai population.

Materials and methods

Patient population

One hundred and six dyspeptic patients who underwent upper gastrointestinal endoscopy at Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, were randomly included in this study. The dyspeptic patients were identified as having non-ulcer dyspepsia (NUD), peptic ulcer dyspepsia (PUD), and gastric cancer (GCA) by the clinical endoscopic doctor and pathologist. During endoscopy, biopsy specimens were taken for the detection of *H. pylori* by culture, rapid urease test (RUT), and histology. Blood samples from each patient were collected in standard vials as described below.

The subjects who had *H. pylori* infection according to our criteria were comprised of 44 NUD, 23 PUD, and 22 GCA. Seventeen subjects (10 NUD, 4 PUD, and 3 GCA) did not have *H. pylori* infection according to our criteria. The patients were 48 males and 58 females with an age range of 18 to 78 years (mean 42 years). Patients who had had antibiotic therapy, as bismuth treatment, proton pump inhibitors, or H_2-blockers within the previous month were excluded.

The positive criteria for *H. pylori* infection were: a positive result for at least one of the invasive methods (i.e., culture, RUT, or histological examination) and positive results by ELISA and Western immunoblot. In contrast, when all invasive methods, the ELISA test, and the Western immunoblot were negative, patients were considered to be *H. pylori*-non-infected.

Control population

A total of 30 consecutive blood donors, asymptomatic for dyspepsia, attending the Srinagarind Hospital Blood Bank, Khon Kaen University, were randomly included. Blood specimens were subjected to ELISA and immunoblot (HB 2.1) analysis.

The blood donors were determined to be in the infected state when ELISA and Western immunoblot were positive, whereas the non-infected state was determined when ELISA and Western immunoblot were negative.

Biopsy specimens and blood samples

Six gastric biopsy specimens (three each from the antrum and the corpus) were taken from each patient for culture, RUT (Pronto Dry test), and histological examination. Blood samples were taken, and sera were separated by centrifugation and stored at − 20 °C until use.

Culture

Culture was performed according to the method of Hazell et al.,[18] with modifications as previously described.[10] Briefly, each antral and corpus specimen was immediately placed into transport medium and brought to the laboratory within 2 h, at 4 °C. Each biopsy specimen was homogenized separately in 200 μl of normal saline solution and cultured on 7% human blood agar (Difco, Detroit, MI, USA) containing the supplement SR147 (5 mg/l trimethoprim, 10 mg/l vancomycin, 5 mg/l amphotericin B, and 5 mg/l cefsulodin; OXOID, Unipath Ltd, Basingstoke, UK). The plates were incubated at 37 °C under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2) and were examined after 4 and 7 days of incubation. Characteristic colonies of *H. pylori* were confirmed by Gram staining, oxidase, catalase, and urease tests.

Commercial rapid urease test (RUT, Pronto Dry test)

The RUT was performed according to the manufacturer's instructions (Medical Instruments Corp., Solothurn, Switzerland) as previously described.[10] Briefly, one antral and one corpus specimen were directly inoculated onto the commercial RUT agar gel. The results were observed and recorded within 24 h. A positive result was indicated when the color changed from yellow to pink.

Histological examination

One antral and one corpus biopsy were fixed in 10% buffered formalin, processed, then embedded in paraffin. Four sections of 3- to 4-μm thick were stained with modified Warthin—Starry stain for identification of *H. pylori*.[19,20] The presence of spiral organisms on any of the slides was considered positive for *H. pylori*.

ELISA and immunoblotting

Serum samples taken from the dyspeptic patients and blood donors were assayed using a commercial ELISA kit (Pyloriset EIA-GIII; Origon, Diagnostica, Espoo, Finland) and a commercial immunoblot assay (Helicoblot 2.1 (HB 2.1); Genelabs Diagnostics, Singapore). ELISA testing was performed according to the manufacturer's recommendations, as published in a previous report.[21]

The immunoblot assay, HB 2.1, was used for the detection of IgG antibodies to specific *H. pylori* proteins derived from the lysates of an ulcerogenic strain and to a recombinant *H. pylori* antigen as the current infection marker (CIM). The assay was performed according to the manufacturer's instructions. Test strips contained different *H. pylori* antigens, including the 116-kDa (CagA), 89-kDa (VacA), 37-kDa, 35-kDa, 30-kDa, and 19.5-kDa antigens, and the CIM. The determination of a positive *H. pylori* infection was made as per the manufacturer's instructions.

The presence of other immunoreactive bands was assessed with a calibrating curve constructed by plotting the distance of migration (in millimeters) of the immunoreactive bands obtained with the calibrating sera against the molecular masses (in kDa) of the corresponding antigens.

Data analysis

The Chi-square and Fisher's exact test were used to compare the presence of specific antigens among patients with different gastroduodenal diseases. Logistic regression analysis was used to determine the risk of gastric cancer. Statistical significance was defined as $p < 0.05$.

Results

The frequency of the immunoreactive bands to CIM, 19.5-, 23-, 26-, 30- (UreA), 35-, 37-, 55-, 66-, 75-, 78-, 85-, 89- (VacA), 93-, 110-, and 116- (CagA) kDa antigens among the *H. pylori*-infected and *H. pylori*-non-infected patient groups, and the *H. pylori*-infected and *H. pylori*-non-infected blood donor groups, are shown in Table 1.

These band antigens were detected more frequently in the *H. pylori*-infected patient group than in the *H. pylori*-non-infected patient group (Table 1). No significant difference in these band antigens was detected in the *H. pylori*-infected patient group compared to the *H. pylori*-infected blood donor group, with the exceptions of the 37-kDa antigen band, which was more frequently observed in the *H. pylori*-infected blood donors than the *H. pylori*-infected patients ($p = 0.024$), and the 89-kDa (VacA) antigen band, which was more frequently observed in the *H. pylori*-infected patients than the *H. pylori*-infected blood donors ($p = 0.039$). The immunoreactive bands were detected in the *H. pylori*-non-infected patients and *H. pylori*-non-infected blood donors less frequently than in the *H. pylori*-infected patients and the *H. pylori*-infected blood donors.

As shown in Table 1, the manufacturer's recommended reference bands at 116-kDa, 89-kDa, 37-kDa, 35-kDa, 30-kDa, 19.5-kDa, and the CIM for *H. pylori*-infected patients had average frequencies of 97.8%, 77.5%, 36.0%, 25.8%, 79.8%, 58.4%, and 69.7%, respectively.

Among the gastroduodenal diseases, seroreactivity to 35-kDa was significantly found in PUD patients ($p = 0.02$) and that to 37-kDa was significantly found in NUD patients ($p = 0.002$). The seroreactivity to 23- and 85-kDa bands was found to be significantly low in PUD patients ($p = 0.01$ and $p = 0.00$, respectively). The seroreactivity to 110-kDa antigen was found to be significantly low in NUD patients ($p = 0.00$). Although the difference did not reach statistical significance, the frequency of 19.5-kDa bands was also found to be higher in GCA patients than in NUD and PUD patients (Figure 1).

Further analysis of the simultaneous combination of immunoreactive bands at 19.5- and 35-kDa in the different gastroduodenal diseases was undertaken; the four phenotypes of antibody patterns are shown in Table 2. Logistic regression analysis showed that the phenotype with the

Table 1 The frequency of *Helicobacter pylori* immunoreactive bands in patients with and without *H. pylori* infection, and in blood donors with and without *H. pylori* infection by ELISA test and Helicoblot 2.1 immunoblotting

Immunoreactive bands (kDa)	Patients		Blood donor group	
	H. pylori-infected (*N* = 89)	*H. pylori*-non-infected (*N* = 17)	*H. pylori*-infected (*N* = 20)	*H. pylori*-non-infected (*N* = 10)
CIM[b]	62 (69.7)	1 (5.9)	17 (85)	1 (10)
19.5[b]	52 (58.4)	3 (17.6)	11 (55)	3 (30)
23	26 (29.2)	1 (5.9)	5 (25)	0 (0)
26	56 (62.9)	3 (17.6)	12 (60)	2 (20)
30 (UreA)[b]	71 (79.8)	2 (11.8)	15 (75)	3 (30)
35[b]	23 (25.8)	0 (0)	7 (35)	1 (10)
37[b]	32 (36.0)[a]	0 (0)	12 (60)[a]	2 (20)
55	55 (61.8)	0 (0)	15 (75)	1 (10)
66	87 (97.8)	17 (100)	20 (100)	10 (100)
75	40 (44.9)	0 (0)	10 (50)	1 (10)
78	29 (32.6)	0 (0)	6 (30)	1 (10)
85	64 (71.9)	0 (0)	11 (55)	2 (20)
89 (VacA)[b]	69 (77.5)[a]	1 (5.9)	11 (55)[a]	2 (20)
93	43 (48.3)	0 (0)	11 (55)	2 (20)
110	75 (84.3)	0 (0)	14 (70)	2 (20)
116 (CagA)[b]	87 (97.8)	9 (52.9)	20 (100)	2 (20)

CIM, current infection marker. Results are *n* (%).
[a] $p < 0.05$.
[b] Molecular weight of *H. pylori* antigen in the strip, according to the manufacturer.

presence of a band at 19.5-kDa combined with the absence of a band at 35-kDa was highly related to the outcome of GCA ($p = 0.007$). The risk of phenotype 2 for GCA was statistically significant with an odds ratio of 3.8, 95% confidence interval of 1.4–10.3 (Table 3).

Discussion

Serological tests are non-invasive methods that have value for the diagnosis of *H. pylori* infection because they are both simple and convenient.[22] One of the serological tests, the immunoblot assay, has the advantage of also being useful to investigate possible relationships between disease presentation and the presence of specific *H. pylori* antigens.[23] Previous studies have demonstrated that immunoblot techniques can reveal multiple major antigens.[5,24–26] Although the serum antibody response to *H. pylori* depends on both the characteristics of the strain and the host response, it can provide clues in predicting the severity of *H. pylori*-associated diseases.

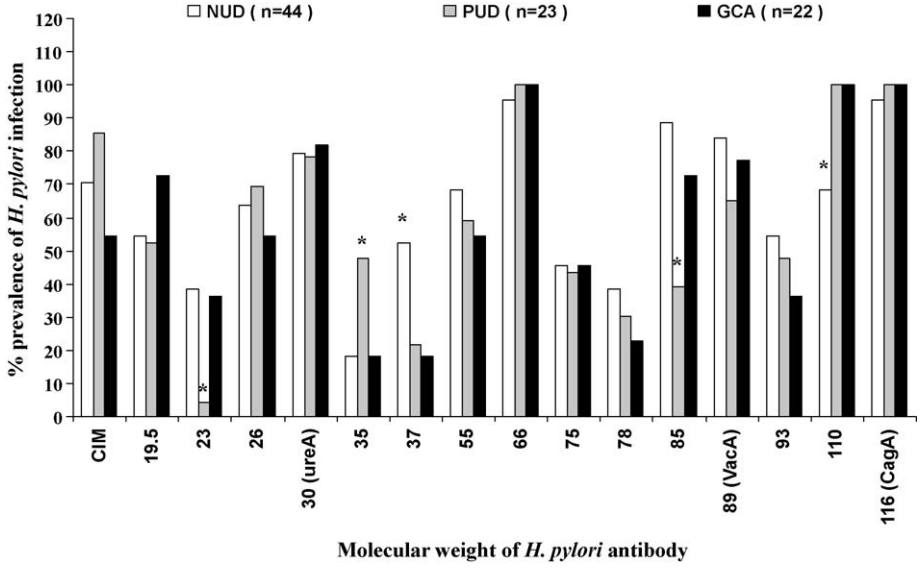

Figure 1 The frequency of *Helicobacter pylori* immunoreactive bands in the gastroduodenal patients. *Statistically significant difference among the gastroduodenal patient groups ($p < 0.05$). NUD, non-ulcer dyspepsia; PUD, peptic ulcer dyspepsia; GCA, gastric carcinoma.

Table 2 The prevalence of antibody patterns using the combination of 19.5-kDa and 35-kDa immunoreactive bands among patients with gastroduodenal diseases

Disease	Total number of patients	No. (%) in the presence or absence of immunoreactive bands (kDa)			
		Phenotype 1 19.5 (+), 35 (+)	Phenotype 2 19.5 (+), 35 (−)	Phenotype 3 19.5 (−), 35 (+)	Phenotype 4 19.5 (−), 35 (−)
Non-ulcer dyspepsia	44	7 (15.9)	16 (36.4)	1 (2.3)	20 (45.5)
Peptic ulcer dyspepsia	23	8 (34.8)	5 (21.7)	3 (13.0)	7 (30.4)
Gastric carcinoma	22	2 (9.1)	14 (63.6)[a]	2 (9.1)	4 (18.2)

[a] $p < 0.05$.

Table 3 The phenotype pattern of immunoreactive bands associated with gastric cancer

Phenotype	Immunoreactive band	Gastric cancer, *n* (%) (*N* = 22)	Non-gastric cancer, *n* (%) (*N* = 67)	*p*-Value	OR (95% CI)
1	19.5 (+), 35 (+)	2 (9.1)	15 (22.4)	0.17	0.35 (<0.005—1.5)
2	19.5 (+), 35 (−)	14 (63.6)	21 (31.3)	0.007	3.8 (1.4—10.3)[a]
3	19.5 (−), 35 (+)	2 (9.1)	4 (6.0)	0.61	1.6 (0.13—8.0)
4	19.5 (−), 35 (−)	4 (18.2)	27 (40.3)	0.06	0.33 (0.11—1.04)

OR, odds ratio; CI, confidence interval.
[a] Statistically significant difference ($p < 0.05$).

In this study, we used HB 2.1 to investigate the anti-*H. pylori* antibody profile in a Thai population in the northeast of Thailand and its putative correlation with the clinical outcome of gastroduodenal diseases. As well as the *H. pylori* standard marker antigens, including 116-, 89-, 37-, 35-, 30-, 19.5-kDa, and CIM defined in the manufacturer's instructions, we also examined other immunoreactive antigens that can be simultaneously detected on the strips, and used these immunoreactive bands for determining any correlation with the clinical outcomes.

Our results show a variety of immunoreactive patterns observed in both *H. pylori*-infected patients and *H. pylori*-infected blood donors, with strong correlations as in previous reports.[5,25] It is not surprising that the immunoreactive patterns from the *H. pylori* infections were similar, because many antigens of *H. pylori* may cross-react with other microorganisms. Moreover, the blood donors may have been infected with *H. pylori*, but were asymptomatic. There was no significant difference in the immunoreactive bands found in the *H. pylori*-infected patients and the *H. pylori*-infected blood donors, with the exceptions of the antibody response against the 37-kDa antigen (significantly higher in the *H. pylori*-infected blood donors than the *H. pylori*-infected patients) and the 89-kDa (VacA) antigen (significantly higher in the overall *H. pylori*-infected patients than the *H. pylori*-infected blood donors).

Although the difference did not reach statistical significance, the frequency of 89-kDa antigen (VacA) was found to be higher in the NUD than in the GCA and PUD groups. This finding agrees with a previous study that demonstrated the anti-VacA antibody was found more frequently in asymptomatic control subjects than in ulcer and gastric cancer groups in France.[27]

CagA and VacA appear to play a major role in pathogenicity.[2,3] The association between the CagA and VacA antibodies and clinical outcomes has been reported.[1,28,29] Both VacA and CagA antigens have been shown to be significantly related to gastroduodenal disease in Western countries,[5,29,30] however no significant association has been found in Asian countries.[12,13,23,31,32] Previous studies have reported that anti-VacA antigen is associated with the presence of inflammation in the gastric body,[33] and that the anti-CagA antigen is highly associated with gastric cancer and peptic ulcer.[34,35] Our findings agree with several Asian studies and a study in a United States population, in which no significant association was found between those antigens and the development of gastroduodenal diseases.[12,13,23,31,32,36] A previous study showed that the antibodies to CagA and 35-kDa antigen were higher in patients with peptic ulcers, especially when they had spent their childhood in Africa.[16] Therefore it might be that not only VacA and CagA, but also the other proteins increase the virulence of disease.

We confirm that VacA and CagA antibodies were not useful to differentiate clinical outcomes in a Thai population. Strong associations between the presence of anti-CagA and the *cagA*-positive and *vacA*s1 *H. pylori* genotypes have been observed.[37] The high prevalences of antibodies to VacA and CagA found in this study correspond to the occurrences of the *vacA* and *cagA* genes in a previous report.[10] The discordance of these reports may be due to geographic variations in *H. pylori* strains or to the host immune response.

We also found that the simultaneous presence of antibody to the 19.5-kDa antigen and the absence of antibody to the 35-kDa antigen of *H. pylori* infection were significantly associated with an increased risk of gastric cancer. This finding agrees with those of previous reports.[17,23] In addition, we confirm the previous observations regarding a relationship between the 35-kDa antigen elicited antibody and peptic ulcer disease.[5,38] However, several immunoreactive bands have been reported to be associated with the

severity of gastroduodenal disease, e.g., the antibodies to the 19.5-, 33- and 136-kDa (CagA) antigen have been shown to indicate a markedly increased probability of more severe gastroduodenal disease.[39] The 33-kDa antigen may be that identified as the 34- or 35-kDa antigen in previous studies[17,23] and in this study. The 34-kDa antigen has recently been identified as OipA, a protein that enhances interleukin-8 production in the host, resulting in cell inflammation and apoptosis.[40]

The 19.5-kDa antigen has been identified as urease accessory protein, UreE, encoded by the *ureE* gene.[41] The role of UreE remains unclear, although it has been hypothesized to provide the active site of urease with nickel ion and to be involved in urease maturation.[42–44] Several studies have suggested that the 35-kDa antigen may play a role in disease pathogenesis and that the 37-kDa antigen of *H. pylori* may be protective. The presence of unidentified factors or antigens that have disease association may exist.[16,17,23]

Other immunoreactive protein bands besides the HB 2.1 marker bands were observed. A low frequency of antibody to 23- and 85-kDa antigens was significantly associated with PUD. The 85-kDa antigen, which may be identical to the 82-kDa antigen reported in a previous study, was identified as putative cytotoxin.[3,45] However, the 22-kDa antigen, which may be 23-kDa in this study, remains to be identified, and proper identification is required to elucidate its nature and role. Future studies are needed to investigate whether the absence of these immunoreactive bands is due to the expression being switched off in response to the gastric environment or whether patients who subsequently develop gastroduodenal diseases are not responding to these antigens.

Our results show a high prevalence of 66-kDa antigen in all *H. pylori*-infected and non-infected patients and donors. The 66-kDa antigen has been identified as UreB.[46,47] Cross-reactivity with antigens of other bacterial species has been shown.[25] The 55-kDa antigen, probably a heat shock protein 54-kDa (*H. pylori* 54-kDa), was also found to be highly prevalent in both infected patients and donors, and has been reported to be increasingly synthesized in response to many environmental stresses.[48] It has been suggested that heat shock proteins might be involved in the pathogenesis of *H. pylori*-related diseases through the induction of an autoimmune response and could be a potential component of a future vaccine.[49]

In this study, seroreactivity to 66-, 110-, and 116-kDa (CagA) antigens were found to be the most prevalent in both *H. pylori*-infected patients and *H. pylori*-infected blood donors. This indicates that these antigens are highly immunogenic. However, seroreactivity to the 35-, 37-, 55-, 75-, 78-, 85-, 93-, and 110-kDa antigens was not found in the *H. pylori*-non-infected patients, therefore these immunoreactive bands should be specific and may be useful for the diagnosis of *H. pylori* infection, and some bands were found more frequently, as in previous reports.[3,25]

Other research groups have frequently found different seroreactive bands for *H. pylori* infection, such as seroreactivity to the 130-, 93-, 75-, and 67-kDa antigens,[50] or to the 110/120-kDa and/or two of five low molecular mass proteins (26-, 29-, 30-, 31-, and 33-kDa, in any combination),[25] and some investigators have found that the 26.5-, 30-, and 116-kDa antigens are the most prevalent.[14–16,25,51] In another report, the 110- and 63-kDa antigens on IgG immunoblot and an 89-kDa antigen on IgA immunoblot were found to be the most specific for *H. pylori* infection.[52] However, Lepper et al.[53] have suggested that the detection of *H. pylori* infection varies because of the use of different criteria for interpreting immunoblot results in *H. pylori* infection.

As well as the use of different criteria for the interpretation of *H. pylori* infection, results also depend on the *H. pylori* strain used for antigen preparation and the polymorphism of the antibody response to *H. pylori*.[5,24–26] Our study used the HB 2.1 strip, which contained different *H. pylori* antigens, and the criteria for a positive *H. pylori* infection were as per the manufacturer's instructions. The other immunoreactive bands were assessed using a calibrating curve. Therefore, prior to the determination of which immunoreactive bands are found, we suggest that the *H. pylori* infection should be determined first and that the molecular weight proteins should be estimated and compared to those of other investigators.

In conclusion, we confirm that the universal antibodies to CagA and VacA are not useful for differentiating clinical outcome among gastroduodenal disease patients in the northeast of Thailand. Using an immunoblot analysis, the simultaneous presence of the 19.5-kDa antigen and the absence of 35-kDa antigen may be useful as an indirect marker for *H. pylori*-infected patients at high risk of gastric cancer. The presence of 35-kDa antigen is associated with peptic ulcer and that of 37-kDa antigen is associated with non-ulcer dyspepsia. Although the cause of gastroduodenal disease, especially gastric cancer, with *H. pylori* infection is multi-factorial, further studies are needed to identify and characterize the pathogenic roles of the 19.5-, 35-, and 37-kDa antigens of *H. pylori*, which may serve as a useful tool in reducing the risk of gastroduodenal diseases. In addition, *H. pylori*-infected patients who have the presence of the 19.5-kDa antigen and the absence of 35-kDa antigen should be followed up for the possible progression of gastric cancer.

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Ethical approval: This study was approved by the Ethics Committee of Khon Kaen University and performed in accordance with the Declaration of Helsinki. Informed consent was obtained from each patient prior to entering the study.

Conflict of interest: No conflict of interest to declare.

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